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Homotypic and Heterotypic IgG and IgM Antibody Responses in Adults Infected With Small Round Structured Viruses

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Antibody responses to recombinant Norwalk (rNV) and Mexico (rMXV) viral capsid proteins were studied in 39 adults involved in outbreaks of gastroenteritis associated with genogroup 2 small round structured viruses (SRSVs). Nineteen individuals were involved in outbreaks associated with MXV-like strains and 20 in outbreaks associated with four other genogroup 2 SRSVs. IgG antibodies were measured in acute and convalescent sera using indirect enzyme-linked immunosorbent assay (ELISA), and IgM was measured by indirect and capture ELISAs. Nineteen (49%) patients demonstrated a significant rise in IgG to rMXV with four (10%) patients also showing anamnestic responses to rNV. Fourteen patients were positive in the rMXV IgM-capture ELISA, representing 74% of patients demonstrating IgG rises. IgG and IgM responses to rMXV were observed in both groups, although higher levels of responses were seen in adults infected with MXV-like strains than those infected with non-MXV genogroup 2 viruses. No significant IgM responses were observed to rNV. These results indicate that, following SRSV infection, adults show a rise in antibody which is broadly reactive to viruses within but not between genogroups, although greater homotypic than heterotypic responses are produced. These findings have implications for interpretation of seroepidemiological studies and serodiagnosis of SRSV infections using recombinant capsids. ***J. Med. Virol. 54:305–312, 1998.***

KEY WORDS: human calicivirus; Norwalk virus; Mexico virus; ELISA; RT-PCR

INTRODUCTION

Small round structured viruses (SRSV) or Norwalk-like viruses are an antigenically diverse group of viruses which are now known to be caliciviruses. These agents cause outbreaks and sporadic cases of gastroenteritis with a particular predilection for producing illness in adults. Diagnosis has largely relied on the demonstration of characteristic virus-like particles in acute faecal samples by electron microscopy (EM) although the demonstration of a fourfold or greater rise in IgG to Norwalk virus between acute and convalescent serum samples has also been used to implicate SRSVs in the aetiology of outbreaks of gastroenteritis [Green et al., 1993].

Reverse transcription polymerase chain reaction (RT-PCR) has been developed for identifying SRSV RNA in faecal samples [Ando et al., 1995; Green et al., 1995b; Jiang et al., 1992a]. This technique has been used for both diagnosis and genetic comparison of antigenically distinct viruses as determined by solid phase EM (SPIEM) [Ando et al., 1994; Norcott et al., 1994]. Such phylogenetic analyses reveal considerable genetic variation within two distinct genogroups [Moe et al., 1994]: Genogroup 1 includes NV, Southampton (SOT), and Desert Shield (DSV) viruses; Genogroup 2 contains Bristol (BRV), Hawaii (HV), MXV, Snow Mountain (SMA), and Melksham (MKV) viruses [Lew et al., 1994a; Green et al., 1994, 1995a].

Radioimmunoassays, blocking enzyme immunoassays (EIA), and biotin-avidin EIAs have been described for the detection of IgG, IgA, and IgM antibodies based on reagents obtained from volunteers challenged with SRSVs [Blacklow et al., 1979; Dolin et al., 1986; Erdman et al., 1989; Greenberg et al., 1978; Madore et al., 1986; Treanor et al., 1988]. More recently, the expres-

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sion of the capsid region of cloned SRSVs in the baculovirus system resulting in the production of virus-like particles has revolutionised the production of high-quality antigen, allowing further development of serological assays [Jiang et al., 1992b]. NV was the first SRSV to be expressed in baculovirus, but recently recombinant capsids have also been produced for DSV, MXV, BRV, and HV [Dingle et al., 1995; Jiang et al., 1995; Lew et al., 1994b; Green et al., 1997].

Studies of serological responses to SRSVs using recombinant capsids indicate that infections result in a rise in serum IgG and IgA antibodies with IgM being observed in the majority of volunteers [Gray et al., 1994; Treanor et al., 1993]. Typically, IgM develops about 1 week following illness [Erdman et al., 1989]. Most symptomatic volunteers have pre-existing IgG antibody, but this does not correlate with susceptibility to infection [Graham et al., 1994]. Resistance to rechallenge with NV has been demonstrated in volunteers, but immunity appears to be short-lived [Wyatt et al., 1974]. Serological assays based on recombinant NV appear more sensitive and specific than those based on human material [Green et al., 1993]. In addition, differences in sensitivities of both IgG and IgM assays are observed between volunteers and patients involved in field outbreaks [Erdman et al., 1989]. Although this phenomenon was observed using assays based on faecal-derived antigen, which may not be the case with recombinant protein, it likely reflects antigenic differences between the infecting strain and the SRSV used in the assay. Homotypic responses are being measured in volunteers, whereas in field outbreaks heterotypic responses are largely being detected.

A number of studies have investigated homotypic and heterotypic responses to SRSVs. Madore and colleagues compared reagents obtained from volunteers challenged with NV, SMA, and HV and demonstrated cross-reactive responses between HV and SMA but less cross-reactivity between these strains and NV [Madore et al., 1990]. Parker studied heterotypic and homotypic SRSV responses in volunteers challenged with NV and in patients involved in SRSV-associated outbreaks using rNV and rMXV capsids [Parker et al., 1995]. Anamnestic responses to rMXV were observed in a proportion of the volunteers demonstrating good responses to rNV and vice versa in patients involved in outbreaks associated with SMA-like viruses. However, some inconsistencies were observed, with patients involved in outbreaks associated with HV-like strains failing to produce rises to rMXV.

Interpretation of studies on serological responses to SRSVs in patients involved in field outbreaks has been hindered by uncertainty in the identity of the infecting SRSV. Indeed, dual infections with distinct SRSVs have been described, which may result in further confusion [Kohn et al., 1995]. The purpose of this study was to investigate homotypic and heterotypic responses to infection with SRSVs in patients involved in outbreaks where the infecting SRSV was available for genetic characterisation. Thus, we were able to compare a panel of paired sera from outbreaks associated with MXV-like viruses with a similarly sized panel of serum samples from patients involved with outbreaks associated with SRSVs genotypically distinct from MXV but belonging to the same genogroup. IgG and IgM responses were measured using assays based on rMXV and rNV capsid proteins.

MATERIALS AND METHODS

Patients

Acute and convalescent serum specimens were retrospectively sought from symptomatic adults involved in outbreaks of gastroenteritis associated with SRSVs. Cases were enrolled into the study where paired sera were received at least 1 week apart, and sufficient SRSV positive faecal material was available from the outbreak to allow RT-PCR and sequence analysis. Cases were recruited from hospital outbreaks and incidents occurring in residential homes for the elderly and included both patients and staff. Nineteen sera were available from three outbreaks of gastroenteritis (RBH/93/UK, AGH/94/UK, and RBH/94/UK) which occurred in the winter of 1993/94 during an epidemic of MXV [Lewis et al., 1997]. Twenty paired sera were also available from cases involved in eight outbreaks of diarrhoea and vomiting which took place between 1990 and 1996.

Genetic Characterisation of SRSV

RT-PCR and amplicon sequencing were performed on SRSV-positive faecal specimens using the primer pair 36/E3 using methods described previously [Hale et al., 1996]. For those strains failing to amplify with 36/E3 the primer pair G2/E3 was used [Gray et al., 1997]. Thus, sequences were available from at least a 231-nucleotide region encoding part of the putative RNA polymerase to allow comparison to published SRSV sequences.

MXV and NV Indirect-IgG and IgM ELISAs

Indirect ELISAs were performed in 96-well U-bottomed microtitre plates (Nunc Immuno Maxisorp, 449824) using methods described by Gray, with some modifications [Gray et al., 1994]. Recombinant capsids were stored at –20°C in aliquots at a concentration of 2 mg/ml prior to use. Recombinant MXV capsids were used to coat plates at a dilution of 1:2,000 in phosphate-buffered saline (PBS; pH 7.4) and rNV capsid protein at a dilution of 1:3,000. All further dilutions were made in PBS containing 1% skim milk. For the IgG assays, doubling serum dilutions were made from 1:100, and a 1:3,000 peroxidase conjugated goat anti-human IgG (Sigma-Aldrich Company Ltd., Poole, Dorset, UK) was used as conjugate. In the IgM assays sera were pretreated to remove IgG and IgA using goat anti-human IgA (Sigma) and goat anti-human IgG (Sigma) both at a ratio of 1:20 (human sera:goat sera). Samples were absorbed by reaction overnight at 4°C and tested at a final dilution of 1:100. A 1:2,000 dilution of peroxidase-conjugated goat anti-human IgM (Sigma) was used in

the final stage of the IgM assays. In all assays, 100 μl of TMB (Europa Research Products, Cambridge, UK) was used as substrate with the reaction being stopped after 20 min by the addition of 100 μl of 1 M sulphuric acid (H_2SO_4). Well optical densities (OD) were measured using the A450/A620 dual wavelength mode of an enzyme-linked immunosorbent assay (ELISA) reader (MCC340 MkII, Wellcome Diagnostics).

MXV and NV capture-IgM ELISAs

ELISA plates were coated with 100 μl of goat anti-human IgM (Tago-immunologicals, Bio-source International, Camarillo, CA) at a dilution of 1:3,000 in 0.01 M carbonate coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$; pH 9.6) and incubated for 4 hr at room temperature. Plates were washed once with PBS containing 0.05% Tween (PBST), blocked by the addition of 200 μl of PBS containing 5% skim milk, and then left overnight at 4°C. Following two washes with PBST, 100 μl of acute and convalescent serum samples diluted at 1:100 in PBS/1% skim milk were added, and the plates were incubated at 37°C for 2 hr. Plates were washed six times, and 100 μl of either rNV or rMXV was added at the same concentrations as used in the indirect ELISAs. Plates were again left overnight at 4°C and washed a further six times. Either 100 μl of 1:10,000 dilution of guinea pig anti-rMXV antibody (MXV assay) or 1:160,000 dilution of rabbit anti-rNV (NV assay) was added, and the plates were incubated at 37°C for 2 hr. Following six washes with PBST, 100 μl of 1:3,000 dilution of peroxidase-conjugated goat anti-guinea pig IgG (Sigma), for the MXV assay, or 100 μl of 1:3,000 dilution of peroxidase-conjugated goat anti-rabbit IgG (Sigma), for the NV assay, both containing 1% normal human serum, were added and incubated for 2 hr at 37°C. After a final six washes, 100 μl of TMB substrate was added to each well, and the reaction stopped and the plates were read as above.

Optimal dilutions of recombinant capsids, hyperimmune animal antisera, and conjugated antibodies were determined prior to testing. Inclusion of 1% normal human sera with the conjugate was found to significantly reduce background in negative control wells, although use of normal goat serum at other stages did not appear to significantly improve the performance of the assays. Duplicate negative and positive controls were included in each plate, which were derived from acute and convalescent sera from a symptomatic volunteer challenged with NV and an adult involved in an outbreak associated with MXV.

Interpretation of ELISAs

Titres in the rMXV and rNV IgG-indirect ELISAs were defined as the highest dilution giving an OD > 0.1. In the indirect and capture-IgM ELISAs convalescent sera were arbitrarily considered positive if OD > 0.1 and the ratio of the OD in the convalescent sera to that of the acute sera (P/N) was >2. This cut-off was adopted as a number of sera showed non-specific reactivity. For samples giving P/N values >2 in the IgM-capture assay, reactivity was shown to be specific as preabsorption with goat anti-human IgM removed or significantly reduced ODs in the ELISA, whereas identical treatment with normal goat serum did not (data not shown).

RESULTS

Patient Demographics

The sex distribution was similar for the two study groups: ten females and seven males for the MXV-like infected group and eight females and four males for the group infected with non–MXV-like strains (information was not available on remaining cases). The MXV-infected group was aged between 26 and 99 with a mean of 74 years, and for the 15 individuals in which age was stated in the non–MXV-like infected group, the age range was 23–91 years with a mean of 56. The difference in age between the two groups was significant ($P = .04$, Student's t-test).

Characterisation of SRSV Strains

Faecal specimens from three outbreaks (RBH/93/UK, AGH/94/UK, and RBH/94/UK) were highly reactive in the rMXV antigen ELISA identifying these as MXV-like strains [Hale et al., 1996; Lewis et al., 1997]. The sequence of AGH/94/UK was available from one of these previous studies [Hale et al., 1996]. Faecal samples from the eight remaining outbreaks were subject to RT-PCR and amplicon sequencing, and the amino acid alignment for this region and the percentage similarity to MXV at the nucleotide level is shown in Figure 1. Phylogenetic analysis revealed that strains clustered into four groups: Strains from two outbreaks (CA/92/UK and SJH/92/UK) were BRV-like [Green et al., 1994], one outbreak (MH/90/UK) was caused by a MKV-like strain [Green et al., 1995a], strains from two outbreaks (Sea/91/UK and WGH/93/UK) were HV-like [Lew et al., 1994a,b], and strains from the remaining three outbreaks (ELH/96, CO/96 and RHCH/96) appeared unique. These strains were most closely related to BRV, exhibiting 92% sequence similarity, but had three amino acid substitutions over this region of ORF 1 (Fig. 1). This strain was first seen associated with an outbreak of gastroenteritis which occurred in Grimsby in 1995 (J. Green, personal communication), and hence, these are referred to as Grimsby-like viruses (GRV). The precise genetic and antigenic relationship of GRV to BRV has yet to be determined. These four genetic clusters align within SRSV genogroup 2, displaying a 70–73% similarity to MXV over a 231-base region of the RNA polymerase gene.

Determination of IgG Responses Using rMXV and rNV Capsids

Results of indirect-IgG ELISAs using MXV and NV recombinant capsids and are shown in Tables I and II. Nineteen (49%) of 39 patients had a fourfold or greater rise in IgG antibody to rMXV. Ten of 19 (53%) were infected with MXV-like viruses (Table I), while nine of 20 (45%) were associated with other SRSV genogroup 2

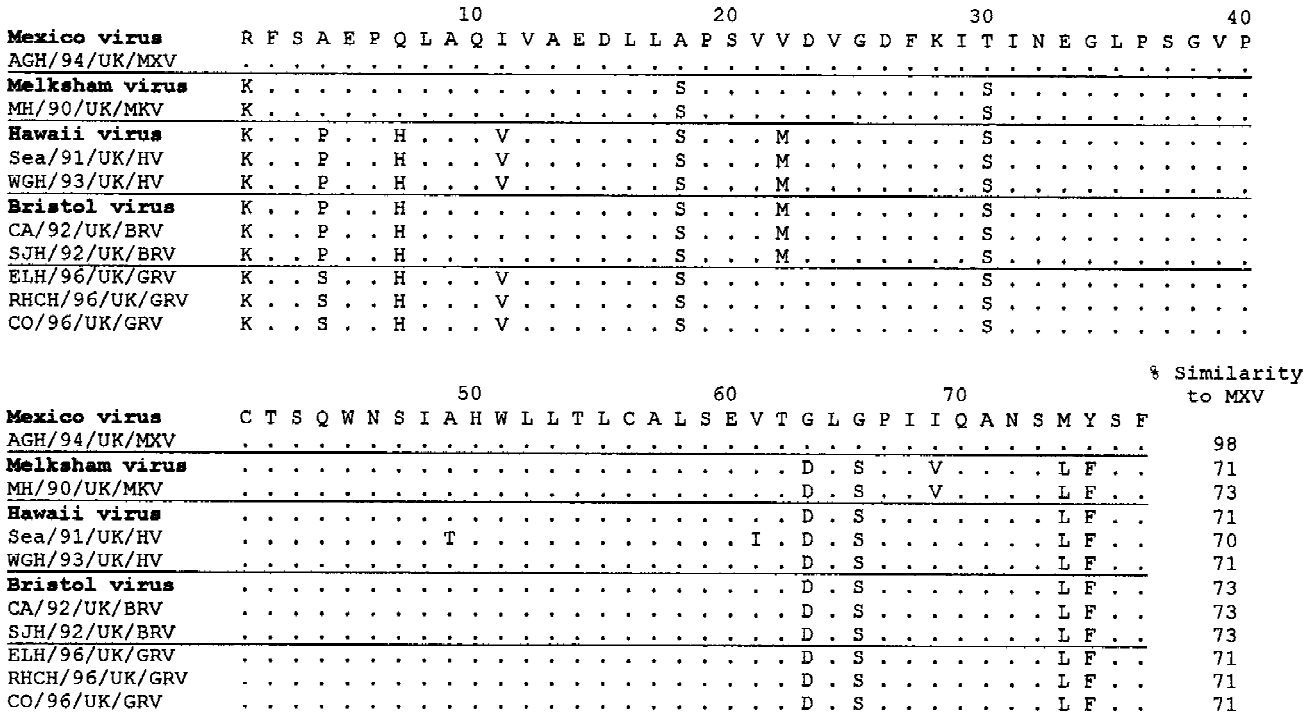

Fig. 1. Amino acid alignment of SRSVs characterised by RT-PCR and direct sequencing of a portion of ORF1 corresponds to locations 4634 to 4864 of NV. Strains are grouped by sequence similarity to previously characterised SRSVs (in bold), and virus notation indicates related prototype. ELH/96/UK, RHCH/96/UK, and CO/96/UK appear to be sufficiently divergent from BRV to classify as a unique genetic cluster (referred to as GRV-like). Percentage nucleotide similarity to MXV is shown.

viruses (Table II). Of these 19 individuals four also showed a fourfold rise in IgG to rNV. IgG rises to rMXV were seen in patients involved in outbreaks associated with strains from all non-MXV genetic clusters. The geometric and arithmetic mean values of acute and convalescent IgG titres to rMXV and rNV were calculated for the MXV-infected group and those infected with other SRSV genogroup 2 strains and compared using the *t*-test (Table III).

Measurement of IgM by Indirect and Capture ELISAs Based on rMXV and rNV

Seven (18%) of 39 patients were positive in the rMXV indirect-IgM ELISA of whom six were infected with MXV-like strains and one was infected with a non-MXV genogroup 2 virus. Six of these seven patients demonstrated a significant rise in IgG titre to rMXV. Fourteen (36%) patients were positive in the rMXV capture-IgM ELISA, which includes all but one patient positive in the rMXV indirect IgM assay. All patients positive by capture-IgM had a fourfold or greater rise in IgG titre to rMXV. Eight patients were infected with MXV-like strains and six with non-MXV genogroup 2 viruses. The mean OD of the convalescent serum of the MXV-infected patients positive in the IgM capture assay was 0.889 compared to 0.265 for the group infected with non-MXV SRSV genogroup 2 viruses.

One patient (15B) was positive in both rNV IgM ELISAs and also in the rMXV indirect-IgM ELISA. However, there was no corresponding rise in IgG to either antigen, suggesting that these were false-positive results. Insufficient sera were available to test for rheumatoid factor or to repeat the indirect-IgM tests following further IgG and IgA absorption. All other specimens tested were negative in the rNV indirect-IgM and capture-IgM ELISAs.

DISCUSSION

Serological assays based on NV have been extensively used for the diagnosis of SRSV-associated outbreaks and for epidemiological studies. However, interpretation of these data has been difficult because of a lack of understanding of homotypic and heterotypic humoral responses to this antigenically diverse group of viruses. The recent cloning and expression of MXV capsid now provides reagents based on a strain which is antigenically and genetically distinct from NV, thus permitting a more thorough investigation of antibody responses following infection with SRSVs. In addition, genogroup 2 SRSVs have been more prevalent in the United Kingdom in recent years.

In this study we compared seroresponses of patients involved in outbreaks of gastroenteritis associated with MXV and patients involved in outbreaks associated with non-MXV genogroup 2 viruses. Fifty percent of patients showed a fourfold or greater rise in IgG to rMXV, and 10% exhibited anamnestic responses to rNV [Parker et al., 1995]. Gray and colleagues demonstrated IgG rises to rNV in all volunteers with symptomatic NV infection [Gray et al., 1993]. The discrep-

TABLE I. Results of 19 Serum Pairs From Patients Involved in Outbreaks of Gastroenteritis Associated With MXV-Like Strains Tested in Indirect and Capture ELISAs Based on Recombinant MXV and NV Capsids*

Outbreak	Case ID	Day[a]	MXV IgG	MXV Cap-IgM	MXV Ind-IgM	NV IgG
AGH/94/UK/MXV (A)	1A	3	3,200	Pos	Neg	12,800
		23	12,800	P/N = 2		25,600
	2A	3	51,200	Neg	Neg	6,400
		23	51,200			3,200
	3A	3	1,600	Neg	Neg	6,400
		23	1,600			6,400
	4A	4	3,200	Pos	Neg	12,800
		24	102,400	P/N = 2.9		25,600
	5A	6	204,800	Neg	Neg	6,400
		23	102,400			6,400
	6A	3	100	Neg	Neg	3,200
		20	25,600			6,400
	7A	2	1,600	POS	POS	100
		28	102,400	P/N = 12	P/N = 23	400
	8A	4	3,200	Neg	Neg	1,600
		33	3,200			3,200
	9A	2	3,200	Neg	Neg	1,600
		30	3,200			1,600
RBH/93/UK/MXV (B)	10B	NK	6,400	POS	Neg	6,400
			102,400	P/N = 3		6,400
	11B	NK	12,800	POS	Pos	6,400
			819,200	P/N = 7	P/N = 2.2	12,800
	12B	NK	1,600	POS	Neg	1,600
			204,800	P/N = 4.9		3,200
	13B	NK	25,600	POS	Pos	6,400
			819,200	P/N = 14	P/N = 3	12,800
	14B	NK	3,200	POS	POS	6,400
			409,600	P/N = 24	P/N = 44	6,400
	15B[b]	3	204,800	Neg	POS	25,600
		11	102,400		P/N = 6	6,400
RBH/94/UK/MXV (C)	16C	7	102,400	Neg	Neg	12,800
		21	51,200			6,400
	17C	7	409,600	Neg	Neg	12,800
		21	409,600			12,800
	18C	7	102,400	POS[c]	POS	400
		21	1,638,400	P/N = 4	P/N = 18	800
	19C	7	6,400	Neg	Neg	51,200
		21	12,800			102,400

*IgG rises ⩾4 are indicated by underlining, and positive IgM values with P/N values between 2 and 3 are indicated by lower case (see Materials and Methods for derivation of P/N). NK, not known.
[a]Number of days after onset of symptoms.
[b]Also positive in NV Cap-IgM and NV Ind-IgM ELISAs.
[c]IgM was present in first serum (OD = 0.7 and rose to 3.0 by day 21).

ancy between their observation and ours deserves further comment. First, faecal specimens were not available from all cases included in this study, and thus, it is possible that a proportion of patients had diarrhoea due to some other cause. Second, there was variability in the timing of collection of acute specimens, and patients may have already been mounting an immune response. This is plausible, as the mean time of collection of acute specimens of cases demonstrating a rise in IgG was 2.3 days compared to 5 days for those failing to show a rise. This difference is significant ($P < .005$, t-test). Finally, many patients in this study were elderly, and their immune responses may not be comparable to those in volunteers who were generally younger.

The proportion of cases showing a significant rise in IgG to rMXV was similar for both the group infected with MXV-like viruses and non-MXV genogroup 2 viruses. Thus, significant heterotypic responses were observed between SRSV strains within genogroup 2. IgG titres to rMXV were significantly higher in the second sera of individuals infected with MXV-like strains that non-MXV genogroup 2 strains. There was also a smaller but significant difference in IgG titres to rMXV in the first serum sample (Table III). However, despite this, the MXV-infected group still had a significantly greater increase in serum IgG titre to rMXV than patients infected with non-MXV genogroup 2 SRSVs (mean rise of 38-fold vs. eightfold, $P = .05$, t-test), indicating greater homotypic than heterotypic IgG responses. The difference in IgG titres to rMXV in the first sera between the two groups may either reflect the lateness in collection of acute samples or the older age of the MXV-infected group. No difference was observed in IgG titres to rNV (Table III), supporting the former hypothesis.

IgM responses were only observed in 18% of the total study group using the rMXV indirect ELISA. The majority of patients had an accompanying rise in IgG titre to rMXV. The individual who failed to show a rise in

TABLE II. Results of 20 Serum Pairs From Patients Involved in Outbreaks of Gastroenteritis Associated With Non-MXV Genogroup 2 SRSVs Tested in Indirect and Capture ELISAs Based on Recombinant MXV and NV Capsids*

Outbreak	Case ID	Day[a]	MXV IgG	MXV Cap-IgM	MXV Ind-IgM	NV IgG
CA/92/UK/BRV	20D	2	800	Neg	Neg	6,400
(D)		9	25,600			25,600
SJH/92/UK/BRV	21E	3	6,400	Neg	Neg	12,800
(E)		12	6,400			12,800
	22E	3	400	Neg	Neg	400
		12	200			200
	23E	3	6,400	Neg	Neg	1,600
		12	6,400			1,600
Sea/91/UK/HV	24F	1	800	Neg	Neg	1,600
(F)		37	12,800			6,400
WGH/93/UK/HV	25G	2	3,200	Neg	Neg	25,600
(G)		18	3,200			51,200
	26G	3	1,600	POS	POS	800
		20	51,200	P/N = 7.5	P/N = 10	1,600
MH/90/UK/MKV	27H	2	6,400	Pos	Neg	6,400
(H)		12	25,600	P/N = 2.9		6,400
	28H	2	3,200	Pos	Neg	400
		12	25,600	P/N = 2.6		400
	29H	11	6,400	Neg	Neg	6,400
		23	6,400			12,800
ELH/96/UK/GRV	30I	7	6,400	Neg	Neg	6,400
(I)		35	6,400			3,200
	31I	5	12,800	Neg	Neg	12,800
		31	25,600			12,800
	32I	5	12,800	Neg	Neg	25,600
		31	25,600			25,600
	33I	11	6,400	Neg	Neg	25,600
		35	12,800			51,200
RHCH/96/UK/GRV	34J	4	3,200	Neg	Neg	400
(J)		39	6,400			800
	35J	1	800	Pos	Neg	12,800
		44	25,600	P/N = 2.5		12,800
CO/96/UK/GRV	36K	4	6,400	Neg	Neg	12,800
(K)		32	6,400			12,800
	37K	5	3,200	Neg	Neg	12,800
		33	25,600			25,600
	38K	1	3,200	Pos	Neg	800
		29	12,800	P/N = 3		3,200
	39K	1	1,600	Neg	Neg	12,800
		29	6,400			12,800

*IgG rises ⩾4 are indicated by underlining, and positive IgM values with P/N values between 2 and 3 are indicated by lower case (see Materials and Methods for derivation of P/N).
[a]Number of days after onset of symptoms.

IgG was also positive in the rNV indirect-IgM ELISA, and this probably represents a false-positive, possibly due to incomplete absorption of IgG and IgA. Unfortunately insufficient sera were available to repeat the absorption step. The relatively low sensitivity of the indirect-IgM assay led us to develop the capture-IgM ELISA, and our results show that this assay detected IgM responses in a greater proportion of SRSV infections; 35% of all patients were positive in the assay based on rMXV. This represents 74% of those demonstrating a rise in IgG to rMXV. The IgM responses observed, however, were relatively poor, with seven of the 13 positives giving P/N values between 2 and 3 (Tables I, II). Low level IgM responses following SRSV infections have also been observed by others and may reflect reinfections or the mucosal nature of the infection [Erdman et al., 1989; Gray et al., 1994; Johnson et al., 1990].

Similar to IgG responses the IgM assays also suggest a greater homotypic response. Only one individual was positive in the indirect IgM assay from the group infected with non-MXV strains in comparison to six of 19 infected with MXV-like strains. In the rMXV capture-IgM ELISA the rate of detection was 25% (5 of 20) in individuals infected with non-MXV genogroup 2 viruses and 47% (9 of 19) with MXV, with generally higher ODs observed in the second sera of the MXV-infected group (although this did not reach statistical significance). The observation of broadly reactive genogroup-specific seroresponses in adults infected with SRSVs supports the classification of SRSVs into two genogroups. Two hypotheses could explain this finding; that the immune response is directed at genogroup-specific epitopes as well as type-specific epitopes; alternatively, infection results in antibody production to SRSVs (within the same genogroup) previously encountered by the individual. This phenomenon is advantageous for serological diagnosis of SRSV infec-

TABLE III. IgG Titres in Acute and Convalescent Sera to rMXV and rNV in Patients Infected With MXV-Like and Non-MXV Genogroup Strains*

	Patients infected with MXV-like strains			Patients infected with other SRSV G2 strains			Difference in arithmetic means and significance[a]
	No. of sera	Arithmetic mean of log titres ± SD	Geometric mean	No. of sera	Arithmetic mean of log titres ± SD	Geometric mean	
rMXV antigen							
First serum	19	4.012 ± 0.94	10,280	20	3.505 ± 0.43	4,620	0.51 (P = .04)
Second serum	19	4.836 ± 0.86	68,550	20	4.017 ± 0.52	15,850	0.82 (P < .001)
Difference in arithmetic means and significance[b]		0.82 (P < .002)			0.51 (P < .001)		
rNV antigen							
First serum	19	3.679 ± 0.63	4,775	20	3.656 ± 0.64	4,530	0.02 (ns)
Second serum	19	3.806 ± 0.55	6,400	20	3.806 ± 0.68	6,400	0
Difference in arithmetic means and significance		0.13 (ns)			0.15 (P = .02)		

*P values determined by the t-test ([a]equal variances, [b]paired samples).
SD, standard deviation; ns, not significant.

tions, as our results would predict that IgG rises to either rMXV or rNV would be observed frequently following infection with either genogroup 2 or genogroup 1 SRSVs, respectively. Conversely, a rise in IgG to a recombinant SRSV capsid protein cannot be construed as evidence of recent infection with homotypic virus. Alternative methods will be required to measure type-specific responses to SRSVs, and peptide mapping is one approach which may be useful in identifying type-specific epitopes.

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